



## Reagentless Real-Time Identification of Individual Microorganisms by BioAerosol Mass Spectrometry (BAMS)

David P. Fergenson<sup>a</sup>, Keith R. Coffee<sup>a</sup>, Maurice E. Pitesky<sup>a</sup>, Herbert J. Tobias<sup>b</sup>, Paul T. Steele<sup>b</sup>, Gregg A. Czerwieniec<sup>b</sup>, Scott C. Russell<sup>b</sup>, Carlton B. Lebrilla<sup>b</sup>, Joanne M. Horn<sup>a</sup>, Matthias Frank<sup>a</sup> and Eric E. Gard<sup>a,\*</sup>  
The Lawrence Livermore National Laboratory, Livermore, CA<sup>a</sup> and The University of California, Davis, CA<sup>b</sup>.

BioAerosol Mass Spectrometry (BAMS) is capable of identifying individual airborne microorganisms in milliseconds at the level of species. BAMS is the hybrid of the established technique of single particle laser mass spectrometry performed under optimal laser conditions with a novel method of data analysis. The combination results in unique mass spectra for different microorganisms that are recognized in real-time and displayed for the user in a continuously updated pie chart. A diagram of the BAMS instrument appears below.

Particles are accelerated into instrument, which is maintained at vacuum, by atmospheric pressure. Each particle is accelerated to a terminal velocity which is a function of its size.

The particle passes through the beams of two continuous wave solid state lasers, scattering light. The light is detected by photomultiplier tubes. The time between the scattering events indicates the particle velocity, and thus size.



2 Solid state continuous wave lasers  
Photomultiplier tubes  
Solid state continuous wave lasers

The velocity of the particle is used to predict its time of arrival at the source of the beam of coaxial laser reflector. Time-of-flight mass spectrometer. A circuit activates the firing of a homogenizer/diverter control Nd-YAG laser operating at 266 nm to disperse and ionize the particle. The ionized ions are collected for real-time analysis where a determination of the particle's identity is made. This process is repeated approximately 100 times per minute.

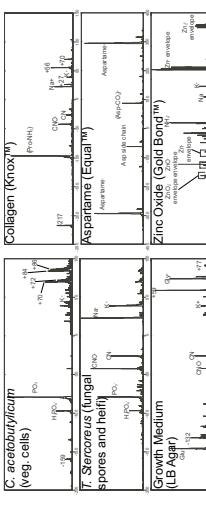
3 The raw spectrum is acquired by the instrument. It is calibrated to read the mass of each bar. The raw spectrum is both externally and internally calibrated.

4 The real-time recognition algorithm is detailed below.

The spectrum is matched with known standard spectra that are expressed in a similar manner by multiplying the one my the other. The dot product will be a match on the scale of 0-1, of their co-linearity, and thus their similarity. Any standard spectrum with a product of 0.7 is a possible match. The analysis is performed independently for both polarities and the best matching negative standard which also matches a positive standard is considered the identity of the spectrum.

5 The real-time recognition algorithm is detailed below.

BAMS can automatically recognize *Bacillus* spores from against a background of many other types of particles. One of BAMS' initial missions was the detection of spores from among suspicious white powders that had been sent in the mail. The six spectra, below, represent samples that may be confused with *Bacillus* spores, *C. acetobutylicum* (veg. cells), *T. Stereocystis* (fungal spores and huffi), *Growth Medium* (LB Agar), *Zinc Oxide (Gold Bond™)*, *Aspartane (Equilin™)*, and *Collagen (Kronex™)*. When analyzed individually, the rate of recognition varied by sample but there were no false positives. The real-time results from analyzing five of the six confounders, *Bacillus* spores, and powdered sugar and baking soda simultaneously are lower right. Note the large "Other" category in response to the 2 unrecognized spores and that fungal spores, which were not added, were not detected.

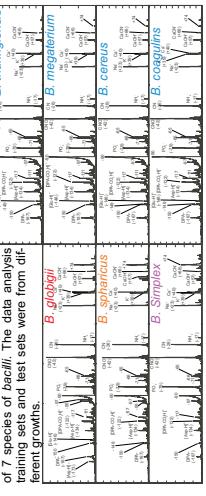


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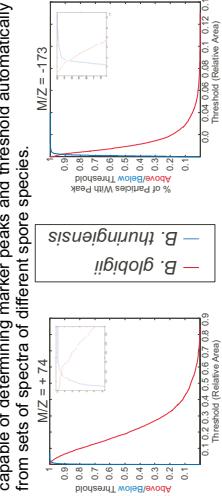


The real-time recognition algorithm is detailed below.

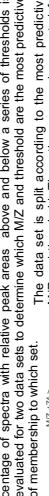
BAMS can determine the species of individual *Bacillus* spores in milliseconds by marker peaks/thresholds. The analysis was capable of accurately identifying the spores as belonging to one of four groups, as noted by the colors of the names, below.



A simple, univariate rules-indicator algorithm, detailed below, is capable of determining marker peaks and threshold automatically from sets of spectra of different spore species.



Averages of 1000 spectra each of four types of clumped viruses.

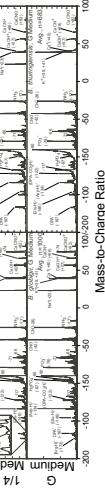


For more about BAMS, please see our other posters at this session:

Poster WPA014-Toward Understanding the Ionization of Biomarkers by Bio-Aerosol Mass Spectrometry, Steele, PT. et al., Submitted to Analytical Chemistry

Laser Power Dependence of Mass Signatures from Individual Bacterial Spores in BioAerosol Mass Spectrometry, Steele, PT. et al., Submitted to Analytical Chemistry

This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. The Lawrence Livermore National Laboratory contributed financially to the experiments through Laboratory Directed Research and Development grant No. 02-ERD-002. This project was also funded by the Technical Support Working Group through the Department of Defense. We would like to express our appreciation to Lawrence Livermore National Laboratory and the Technical Support Working Group for sponsoring this research effort.



Or read our upcoming papers:

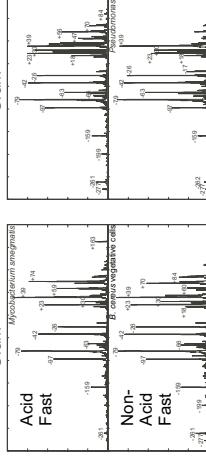
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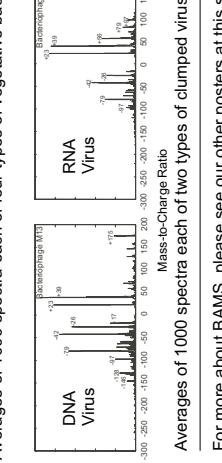
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We are pushing BAMS beyond *Bacillus* spores into other types of microorganisms. We have analyzed Gram- and Gram+ bacteria, both acid fast and non-acid fast, DNA and RNA viruses and fungi. We are in the process of applying our algorithms to these organisms.



Averages of 1000 spectra each of four types of vegetative bacteria.

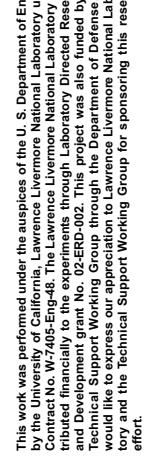


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